Exhibit 5

50875/JPW/AKC

Application for United States Letters Patent

To all whom it may concern:

Be it known that Graham P.Allaway, Virginia M. Litwin, Paul J. Maddon and William C. Olson

have invented certain new and useful improvements in

A METHOD FOR PREVENTING HIV-1 INFECTION OF $CD4^+$ CELLS

of which the following is a full, clear and exact description.

Applicants: William C. Olson, et al.
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Exhibit 5

A METHOD FOR PREVENTING HIV-1 INFECTION OF CD4$^+$ CELLS

Background of the Invention

Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found at the end of this application, preceding the claims.

Chemokines are a family of related soluble proteins of molecular weight between 8 and 10KDa, secreted by lymphocytes and other cells, which bind receptors on target cell surfaces resulting in the activation and mobilization of leukocytes, for example in the inflammatory process. Recently, Cocchi et al. demonstrated that the chemokines RANTES, MIP-1α and MIP-1β are factors produced by CD8$^+$ T lymphocytes which inhibit infection by macrophage-tropic primary isolates of HIV-1, but not infection by laboratory-adapted strains of the virus (1). These chemokines are members of the C-C group of chemokines, so named because they have adjacent cysteine residues, unlike the C-X-C group which has a single amino acid separating these residues (2). While Cocchi et al. found that expression of HIV-1 RNA was suppressed by treatment with the chemokines, they did not identify the site of action of these molecules.

A resonance energy transfer (RET) assay of HIV-1 envelope glycoprotein-mediated membrane fusion was used to determine whether fusion mediated by the envelope glycoprotein from the primary macrophage-tropic isolate

of HIV-1_{JR-FL} would be specifically inhibited by chemokines, when compared with fusion mediated by the envelope glycoprotein from the laboratory-adapted T lymphotropic strain HIV-1_{LAI}. As described below, it was demonstrated that this is indeed the case. This demonstrates that some chemokine receptors are fusion accessory molecules required for HIV-1 infection. Previous studies have indicated that unidentified cell surface molecules are required for virus entry in addition to the HIV-1 receptor, CD4. While CD4 is required for HIV-1 attachment, the accessory molecules are required for the membrane fusion step of entry. These accessory molecules are generally expressed only on human cells, so HIV-1 does not infect non-human $CD4^+$ cells (3-6). Moreover it is possible to complement non-human $CD4^+$ cells by fusing them (using polyethylene glycol) with $CD4^-$ human cells, resulting in a heterokaryon which is a competent target for HIV-1 envelope-mediated membrane fusion (7,8). These studies have been performed using laboratory-adapted T lymphotropic strains of the virus.

In some cases, it appears that fusion accessory molecules are found on a subset of human $CD4^+$ cells and are required for infection by HIV-1 isolates with particular tropisms. For example, macrophage-tropic primary strains of HIV-1 such as HIV-1_{JR-FL} may have different requirements for accessory molecules compared with laboratory-adapted T lymphotropic strains such as HIV-1_{LAI}. This phenomenon may explain differences in tropism between HIV-1 strains.

The current invention comprises a series of new therapeutics for HIV-1 infection. It was demonstrated for the first time that chemokines act at the fusion step of HIV-1 entry and specifically inhibit membrane fusion mediated by the envelope glycoprotein of primary

macrophage-tropic primary viral isolates, not laboratory-adapted T lymphotrophic strains of the virus. Primary macrophage-tropic isolates of the virus are of particular importance since they are the strains usually involved in virus transmission, and may have particular importance in the pathogenesis of HIV-1 infection.

These results were obtained using a resonance energy transfer (RET) assay of HIV-1 envelope-mediated membrane fusion. Moreover, this assay is used to identify non-chemokines, including fragments of chemokines and modified chemokines, that inhibit HIV-1 envelope glycoprotein-mediated membrane fusion and thereby neutralize the virus, yet do not induce an inflammatory response.

Summary of the Invention

This invention provides a method for preventing fusion of HIV-1 to CD4$^+$ cells which comprises contacting CD4$^+$ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of HIV-1 to the CD4$^+$ cells is prevented.

This invention also provides a method for preventing HIV-1 infection of CD4$^+$ cells which comprises contacting CD4$^+$ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of HIV-1 to the CD4$^+$ cells is prevented, thereby preventing the HIV-1 infection.

This invention further provides non-chemokine agents capable of binding to the chemokine receptor and preventing fusion of HIV-1 to CD4$^+$ cells.

In addition, this invention provides pharmaceutical compositions comprising an amount of such non-chemokine agents effective to prevent fusion of HIV-1 to CD4$^+$ cells and a pharmaceutically acceptable carrier.

This invention provides a composition of matter capable of binding to the chemokine receptor and preventing fusion of HIV-1 to CD4$^+$ cells comprising a non-chemokine agent linked to a ligand capable of binding to a cell surface receptor of the CD4$^+$ cells other than the chemokine receptor such that the binding of the non-chemokine agent to the chemokine receptor does not prevent the binding of the ligand to the other receptor.

This invention also provides a pharmaceutical composition comprising an amount of the above-described composition

of matter effective to prevent fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

This invention provides a composition of matter capable of binding to the chemokine receptor and preventing fusion of HIV-1 to $CD4^+$ cells comprising a non-chemokine agent linked to a compound capable of increasing the *in vivo* half-life of the non-chemokine agent.

This invention also provides a pharmaceutical composition comprising an amount of a composition of matter comprising a non-chemokine agent linked to a compound capable of increasing the *in vivo* half-life of the non-chemokine agent effective to prevent fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

This invention provide methods for preventing HIV-1 infection in a subject comprising administering an above-described pharmaceutical composition to the subject. This invention also provides methods for treating HIV-1 infection in a subject comprising administering an above-described pharmaceutical composition to the subject.

This invention also provides methods for determining whether a non-chemokine agent is capable of inhibiting the fusion of HIV-1 to a $CD4^+$ cell which comprise: (a) contacting (i) a $CD4^+$ cell which is labeled with a first dye and (ii) a cell expressing the HIV-1 envelope glycoprotein on its surface which is labeled with a second dye, in the presence of an excess of the agent under conditions permitting the fusion of the $CD4^+$ cell to the cell expressing the HIV-1 envelope glycoprotein on its surface in the absence of the agent, the first and second dyes being selected so as to allow resonance energy transfer between the dyes; (b) exposing the

product of step (a) to conditions which would result in resonance energy transfer if fusion has occurred; and (c) determining whether there is a reduction of resonance energy transfer, when compared with the resonance energy transfer in the absence of the agent, a decrease in transfer indicating that the agent is capable of inhibiting fusion of HIV-1 to $CD4^+$ cells.

Brief Description of the Figures

Figure 1. **Membrane fusion mediated by the HIV-1_{JR-FL} envelope glycoprotein is inhibited by RANTES, MIP-1α and MIP-1β.**

%RET resulting from the fusion of PM1 cells and HeLa-env_{JR-FL} (■) or HeLa-env_{LAI} (◆) was measured in the presence and absence of recombinant human chemokines at a range of concentrations: RANTES (80 - 2.5 ng/ml), MIP-1α (400 - 12.5 ng/ml) and MIP-1β (200 - 6.25 ng/ml), as indicated. Chemokines were added simultaneously with the cells at the initiation of a four hour incubation. Data are representative of more than three independent experiments which were run in duplicate. The percent inhibition of RET is defined as follows:

% Inhibition = 100 . [(Max RET - Min RET) - (Exp RET- Min RET)]/(Max RET - Min RET)

where Max RET is the %RET value obtained at four hours with HeLa-env cells and CD4-expressing cells in the absence of an inhibitory compound; Exp RET is the %RET value obtained for the same cell combination in the presence of an inhibitory compound and Min RET is the background %RET value obtained using HeLa cells in place of HeLa envelope-expressing cells.

Figure 2. **CD4:HIV-1 gp120 binding in the presence of**

human chemokines.

The binding of soluble human CD4 to HIV-1_{LAI} and HIV-1_{JR-FL} gp120 was determined in an ELISA assay in the presence and absence of the monoclonal antibody OKT4A or recombinant human chemokines at a range of concentrations, identical to those used in the RET inhibition studies of Figure 1: OKT4A (62 - 0.3 nM), RANTES (10.3 - 0.3 nM), MIP-1α (53.3 - 2.9 nM), and MIP-1β (25.6 - 0.8 nM). Inhibitors were added simultaneously with biotinylated HIV-1 gp120 to soluble CD4 coated microtiter plates (Dynatech Laboratories, Inc., Chantilly, VA). Following a two hour incubation at room temperature and extensive washing, an incubation with streptavidin-horseradish peroxidase was performed for one hour at room temperature. Following additional washes, substrate was added and the OD at 492 nm determined in an ELISA plate reader. Data are representative of two independent experiments which were run in quadruplicate.

Detailed Description of the Invention

This invention provides a method for preventing fusion of HIV-1 to $CD4^+$ cells which comprises contacting $CD4^+$ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of HIV-1 to the $CD4^+$ cells is prevented.

This invention also provides a method for preventing HIV-1 infection of $CD4^+$ cells which comprises contacting $CD4^+$ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of HIV-1 to the $CD4^+$ cells is prevented, thereby preventing the HIV-1 infection.

In this invention, a chemokine means RANTES, MIP-1-α, MIP-1-β or another chemokine which blocks HIV-1 infection. A chemokine receptor means a receptor capable of lending RANTES, MIP-1-α, MIP-1-β or another chemokine which blocks HIV-1 infection.

The HIV-1 used in this application unless specified will mean clinical or primary or field isolates or HIV-1 viruses which maintain their clinical characteristics. The HIV-1 clinical isolates may be passaged in primary peripheral blood mononuclear cells. The HIV-1 clinical isolates may be macrophage-trophic.

The non-chemokine agents of this invention are capable of binding to chemokine receptors and preventing fusion of HIV-1 to $CD4^+$ cells. The non-chemokine agents include, but are not limited to, chemokine fragments and chemokine derivatives, but do not include naturally occurring chemokines.

In an embodiment of this invention, the non-chemokine agent is an oligopeptide. In another embodiment, the non-chemokine agent is a polypeptide. In still another embodiment, the non-chemokine agent is an antibody or a portion thereof. Antibody against the chemokine receptor may easily be generated by routine experiments. It is also within the level of ordinary skill to synthesize fragments of the antibody capable of binding to the chemokine receptor. In a further embodiment, the non-chemokine agent is a nonpeptidyl agent.

This invention provides non-chemokine agents capable of binding to the chemokine receptor and preventing fusion of HIV-1 to $CD4^+$ cells.

This invention also provides pharmaceutical compositions comprising an amount of such non-chemokine agents effective to prevent fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

Pharmaceutically acceptable carriers are well known to those skilled in the art. Such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present,

such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like.

This invention provides a composition of matter capable of binding to the chemokine receptor and preventing fusion of HIV-1 to $CD4^+$ cells comprising a non-chemokine agent linked to a ligand capable of binding to a cell surface receptor of the $CD4^+$ cells other than the chemokine receptor such that the binding of the non-chemokine agent to the chemokine receptor does not prevent the binding of the ligand to the other receptor. In an embodiment, the cell surface receptor is CD4. In another embodiment, the ligand is an antibody or a portion of an antibody.

This invention also provides a pharmaceutical composition comprising an amount of an above-described composition of matter effective to prevent fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

This invention provides a composition of matter capable of binding to the chemokine receptor and preventing fusion of HIV-1 to $CD4^+$ cells comprising a non-chemokine agent linked to a compound capable of increasing the *in vivo* half-life of the non-chemokine agent. In an embodiment, the compound is polyethylene glycol.

This invention also provides a pharmaceutical composition comprising an amount of a composition of matter comprising a non-chemokine agent linked to a compound capable of increasing the *in vivo* half-life of the non-chemokine agent effective to prevent fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

This invention provide methods for preventing HIV-1

infection in a subject comprising administering the above-described pharmaceutical compositions to the subject. This invention also provides methods for treating HIV-1 infection in a subject comprising administering the above-described pharmaceutical compositions to the subject.

This invention also provides methods for determining whether a non-chemokine agent is capable of inhibiting the fusion of HIV-1 to a $CD4^+$ cell which comprise: (a) contacting (i) a $CD4^+$ cell which is labeled with a first dye and (ii) a cell expressing the HIV-1 envelope glycoprotein on its surface which is labeled with a second dye, in the presence of an excess of the agent under conditions permitting the fusion of the $CD4^+$ cell to the cell expressing the HIV-1 envelope glycoprotein on its surface in the absence of the agent, the first and second dyes being selected so as to allow resonance energy transfer between the dyes; (b) exposing the product of step (a) to conditions which would result in resonance energy transfer if fusion has occurred; and (c) determining whether there is a reduction of resonance energy transfer, when compared with the resonance energy transfer in the absence of the agent, a decrease in transfer indicating that the agent is capable of inhibiting fusion of HIV-1 to $CD4^+$ cells.

HIV-1 only fuses with appropriate $CD4^+$ cells. For example, laboratory-adapted T lymphotropic HIV-1 strains fuse with most $CD4^+$ human cells. Clinical HIV-1 isolates do not fuse with most transformed $CD4^+$ human cell lines but do fuse with human primary $CD4^+$ cells such as $CD4^+$ T lymphocytes and macrophages. Routine experiments may be easily performed to determine whether the $CD4^+$ cell is appropriate for the above fusion assay.

As described in this invention, the HIV-1 membrane fusion is monitored by a resonance energy transfer assay. The assay was described in the International Application Number, PCT/US94/14561, filed December 16, 1994 with International Publication Number WO 95/16789. This assay is further elaborated in a United States co-pending application no. 08/475,515, filed June 7, 1995. The contents of these applications are hereby incorporated by reference into this application.

In an embodiment of the above method, the non-chemokine agent is an oligopeptide. In another embodiment, the non-chemokine agent is a polypeptide. In still another embodiment, the agent is an antibody or a portion thereof. In a further embodiment, the non-chemokine agent is a nonpeptidyl agent.

In a separate embodiment, the $CD4^+$ cell is a PM1 cell. In another embodiment, the cell expressing the HIV-1 envelope glycoprotein is a HeLa cell expressing $HIV\text{-}1_{JR\text{-}FL}$ gp120/gp41.

This invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

1) Chemokines inhibit fusion mediated by the envelope glycoprotein from a macrophage-tropic primary isolate of HIV-1 but not from a laboratory-adapted T-lymphotrophic strain of the virus

The chemokines RANTES, MIP-1α and MIP-1β were obtained from R & D systems (Minneapolis, MN). They were tested in the RET assay for ability to inhibit fusion between HeLa-env_{JR-FL} cells (expressing gp120/gp41 from the macrophage tropic isolate HIV-1_{JR-FL}) and PM1 cells, or for inhibition of fusion between HeLa-env_{LAI} cells (expressing gp120/gp41 from the laboratory-adapted strain HIV-1_{LAI}) and various $CD4^+$ T lymphocyte cell lines. As shown in Figure 1, all three chemokines inhibited fusion mediated by the macrophage tropic virus envelope glycoprotein, but not that mediated by the laboratory-adapted strain envelope glycoprotein.

The ability of the chemokines to block the interaction between CD4 and HIV-1 gp120 which occurs at virus attachment was then tested. It was found that the chemokines did not inhibit this interaction (Figure 2), demonstrating that their blockade of HIV-1 envelope glycoprotein-mediated membrane fusion occurs at the membrane fusion event itself, rather than the initial CD4-gp120 interaction which precedes fusion.

2) Non-chemokine peptides and derivatives that inhibit HIV-1 fusion

The non-chemokines include chemokine fragments and chemokine derivatives that are tested in the RET assay to determine which are active in inhibiting HIV-1 membrane

fusion. Particular attention is focused on fragments or derivatives that inhibit HIV-1 fusion but do not activate leukocyte responses. These non-chemokines include:

a) N-terminal derivatives of the chemokines. Addition of residues to the N-terminus of chemokines inhibits the function of these proteins without significantly reducing their ability to bind chemokine receptors. For example, Met-RANTES (RANTES with an N-terminal methionine) has been shown to be a powerful antagonist of native RANTES and is unable to induce chemotaxis or calcium mobilization in certain systems. The mechanism of antagonism appears to be competition for receptor binding (9). Similar results were found using other derivatives of the N terminus of RANTES(9) and also by N-terminal modification of other chemokines, such as IL-8 (a member of the C-X-C chemokines) (10). The current invention includes Met-RANTES and other chemokines derivatised by the addition of methionine, or other residues, to the N-terminus so that they inhibit fusion mediated by the envelope glycoprotein of HIV-1_{JR-FL}, and inhibit infection by many isolates of HIV-1, yet do not activate the inflammatory response.

b) Chemokines with N-terminal amino acids deleted: Chemokine antagonists have been generated by deleting amino acids in the N-terminal region. For example, deletion of up to 8 amino acids at the N-terminus of the chemokine MCP-1 (a member of the C-C chemokine group), ablated the bioactivity of the protein while allowing it to retain chemokine receptor binding and the ability to inhibit activity of native MCP-1 (11,12).

The current invention includes N-terminal deletants of RANTES, MIP-1α and MIP-1β, lacking the biological

activity of the native proteins, which inhibit HIV-1 fusion and HIV-1 infection.

c) Other peptides: A series of overlapping peptides (e.g. of 20-67 residues) from all regions of RANTES, MIP-1α and MIP-1β are screened by the same approaches to identify peptides which inhibit HIV-1 fusion most potently without activating leukocytes. Activation of leukocyte responses is measured following routine procedures (9, 10, 11, 12).

3) Cloning the chemokine receptors

Chemokine receptors required for HIV-1 fusion are cloned by the following strategy. First a cDNA library is made in a mammalian expression vector (e.g. pcDNA3.1 from Invitrogen Corp. San Diego, CA) using mRNA prepared from the PM1 cell line or $CD4^+$ T-lymphocytes or macrophages. Degenerate oligonucleotide probes are used to identify members of the cDNA library encoding members of the chemokine receptor family, for example following previously published methods (2). The vectors containing chemokine receptor cDNAs are then individually expressed in one of several mammalian cell lines which express human CD4 but do not fuse with HeLa-env_{JR-FL} cells (e.g. HeLa-CD4, CHO-CD4 or COS-CD4) or HeLa-env_{LAI} cells (e.g. CHO-CD4 or COS-CD4). Following analysis in the RET assay, clones which gain the ability to fuse with HeLa-env_{JR-FL} or HeLa-env_{LAI} are identified and the coding sequences recovered, for example by PCR amplification, following procedures well known to those skilled in the art. DNA sequencing is then performed to determine whether the cDNA recovered encodes a known chemokine receptor. Following expression of the receptor, monoclonal and polyclonal antibodies are prepared and tested for ability to inhibit infection by a panel of HIV-1 isolates.

References

1. Cocchi, F., DeVico, A. L., Garzino-Demo, A., Arya, S. K., Gallo, R. C., Lusso, P. 1995. *Science*. 270:1811-1815.

2. Raport, C. J., Schweickart, V. L., Chantry, D., Eddy Jr., R. L., Shows, T. B., Godiska, R., Gray, P. W. 1996. *Journal of Leukocyte Biology*. 59: 18-23.

3. Maddon PJ., Dalgleish AG., McDougal JS., Clapham PR., Weiss RA., Axel R. 1986. *Cell*. 47:333-348.

4. Ashorn PA., Berger EA., Moss B. 1990. *J. Virol*. 64:2149-2156.

5. Clapham PR., Blanc D., Weiss RA. 1991. *Virology*. 181:703-715.

6. Harrington RD., Geballe AP. 1993. *J. Virol*. 67:5939-5947.

7. Broder CC., Dimitrov DS., Blumenthal R., Berger EA. 1993. *Virology*. 193:483-491.

8. Dragic T., Charneau P., Clavel F., Alizon M. 1992. *J. Virol*. 66:4794-4802.

9. Wells, T. N., Power, C. A., Lusti-Narasimhan, M., Hoogewerf, A. J., Cooke, R. M., Chung, C. W., Peitsch, M. C., Proudfoot, A. E. 1996. *Journal of Leukocyte Biology*. 59:53-60.

10. Moser, B., Dewald, B., Barella, L., Schumacher, C., Baggiolini, M., Clark-Lewis, I. 1993. *Journal of*

Biological Chemistry. 268:7125-7128.

11. Gong, J. H., Clark-Lewis, I. 1995. *J. Exp. Med.* 181:631-640.

12. Zhang, Y. J., Rutledge, B. J., Rollins, B. J. 1994. *Journal of Biological Chemistry*. 269:15918-15924.

What is claimed is:

1. A method for preventing fusion of HIV-1 to $CD4^+$ cells which comprises contacting $CD4^+$ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of HIV-1 to the $CD4^+$ cells is prevented.

2. A method for preventing HIV-1 infection of $CD4^+$ cells which comprises contacting $CD4^+$ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of HIV-1 to the $CD4^+$ cells is prevented, thereby preventing HIV-1 infection.

3. The method of claim 1 or 2, wherein the non-chemokine agent is an oligopeptide.

4. The method of claim 1 or 2, wherein the non-chemokine agent is a polypeptide.

5. The method of claim 1 or 2, wherein the non-chemokine agent is an antibody or a portion of an antibody.

6. The method of claim 1 or 2, wherein the non-chemokine agent is a nonpeptidyl agent.

7. A non-chemokine agent capable of binding to the chemokine receptor and preventing fusion of HIV-1 to $CD4^+$ cells.

8. A pharmaceutical composition comprising an amount of the non-chemokine agent of claim 7 effective to prevent fusion of HIV-1 to $CD4^+$ cells and a

pharmaceutically acceptable carrier.

9. A composition of matter capable of binding to the chemokine receptor and preventing fusion of HIV-1 to $CD4^+$ cells comprising a non-chemokine agent linked to a ligand capable of binding to a cell surface receptor of the $CD4^+$ cells other than the chemokine receptor such that the binding of the non-chemokine agent to the chemokine receptor does not prevent the binding of the ligand to the other receptor.

10. The composition of matter of claim 9, wherein the cell surface receptor is CD4.

11. The composition of matter of claim 9, wherein the ligand comprises an antibody or a portion of an antibody.

12. A pharmaceutical composition comprising an amount of the composition of matter of claim 9 effective to prevent fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

13. A composition of matter capable of binding to the chemokine receptor and preventing fusion of HIV-1 to $CD4^+$ cells comprising a non-chemokine agent linked to a compound capable of increasing the *in vivo* half-life of the non-chemokine agent.

14. The composition of matter of claim 13, wherein the compound is polyethylene glycol.

15. A pharmaceutical composition comprising an amount of the composition of claim 13 effective to prevent fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically

acceptable carrier.

16. A method for preventing HIV-1 infection in a subject comprising administering the pharmaceutical composition of claim 8, 12 or 15 to the subject.

17. A method for treating HIV-1 infection in a subject comprising administering the pharmaceutical composition of claim 8, 12 or 15 to the subject.

18. A method for determining whether a non-chemokine agent is capable of inhibiting the fusion of HIV-1 to a $CD4^+$ cell which comprises:

(a) contacting (i) a $CD4^+$ cell, which is labeled with a first dye, with (ii) a cell expressing the HIV-1 envelope glycoprotein on its surface, which is labeled with a second dye, in the presence of an excess of the agent under conditions permitting the fusion of the $CD4^+$ cell to the cell expressing the HIV-1 envelope glycoprotein on its surface in the absence of the agent, the first and second dyes being selected so as to allow resonance energy transfer between the dyes;

(b) exposing the product of step (a) to conditions which would result in resonance energy transfer if fusion has occurred; and

(c) determining whether there is a reduction of resonance energy transfer, when compared with the resonance energy transfer in the absence of the agent, a decrease in transfer indicating that the agent is capable of inhibiting fusion of HIV-1 to $CD4^+$ cells.

19. The method of claim 18, wherein the agent is an

oligopeptide.

20. The method of claim 18, wherein the agent is a polypeptide.

21. The method of claim 18, wherein the agent is an antibody or a portion of an antibody.

22. The method of claim 18, wherein the agent is a nonpeptidyl agent.

23. The method of claim 18, wherein the $CD4^+$ cell is a PM1 cell.

24. The method of claim 18, wherein the cell expressing the HIV-1 envelope glycoprotein is a HeLa cell expressing $HIV\text{-}1_{JR\text{-}FL}$ gp120/gp41.

A METHOD FOR PREVENTING HIV-1 INFECTION OF CD4$^+$ CELLS

Abstract of the Disclosure

This invention provides methods for preventing fusion of HIV-1 to CD4$^+$ cells which comprise contacting CD4$^+$ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of HIV-1 to the CD4$^+$ cells is prevented. This invention also provides methods for preventing HIV-1 infection of CD4$^+$ cells which comprise contacting CD4$^+$ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of HIV-1 to the CD4$^+$ cells is prevented, thereby preventing the HIV-1 infection. This invention provides non-chemokine agents capable of binding to the chemokine receptor and preventing fusion of HIV-1 to CD4$^+$ cells. This invention also provides pharmaceutical compositions comprising an amount of the non-chemokine agent capable of binding to the chemokine receptor and preventing fusion of HIV-1 to CD4$^+$ cells effective to prevent fusion of HIV-1 to CD4$^+$ cells and a pharmaceutically acceptable carrier.

FIG. 1A

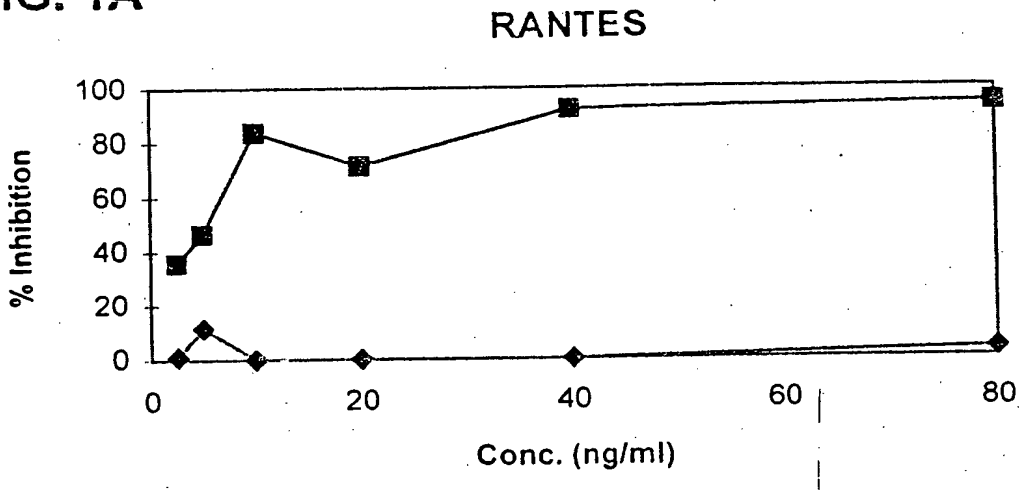


FIG. 1B

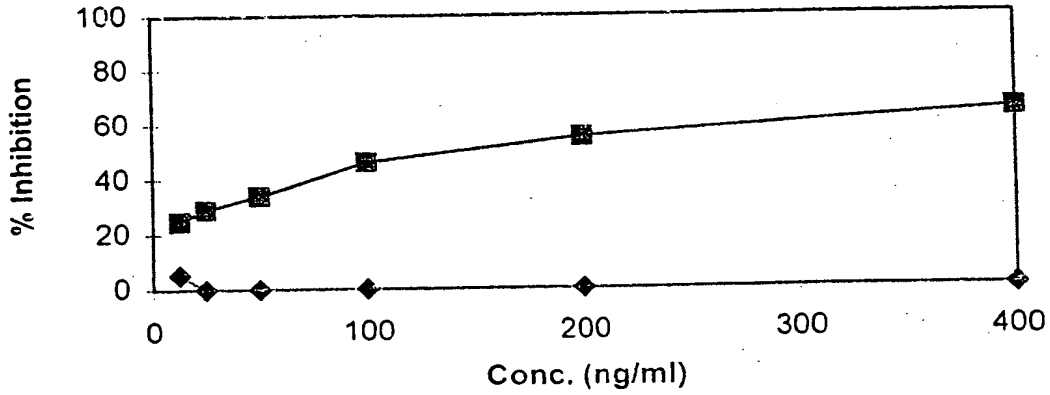


FIG. 1C

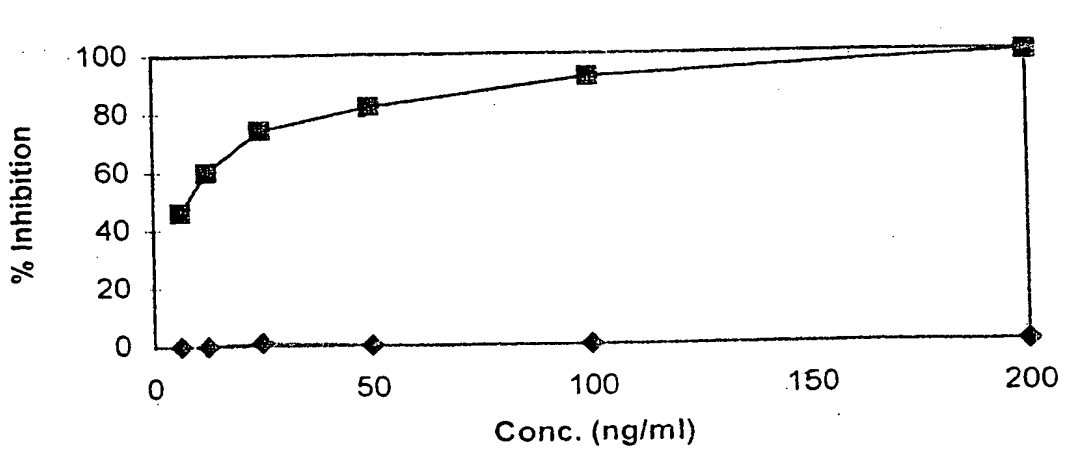


FIG. 2A

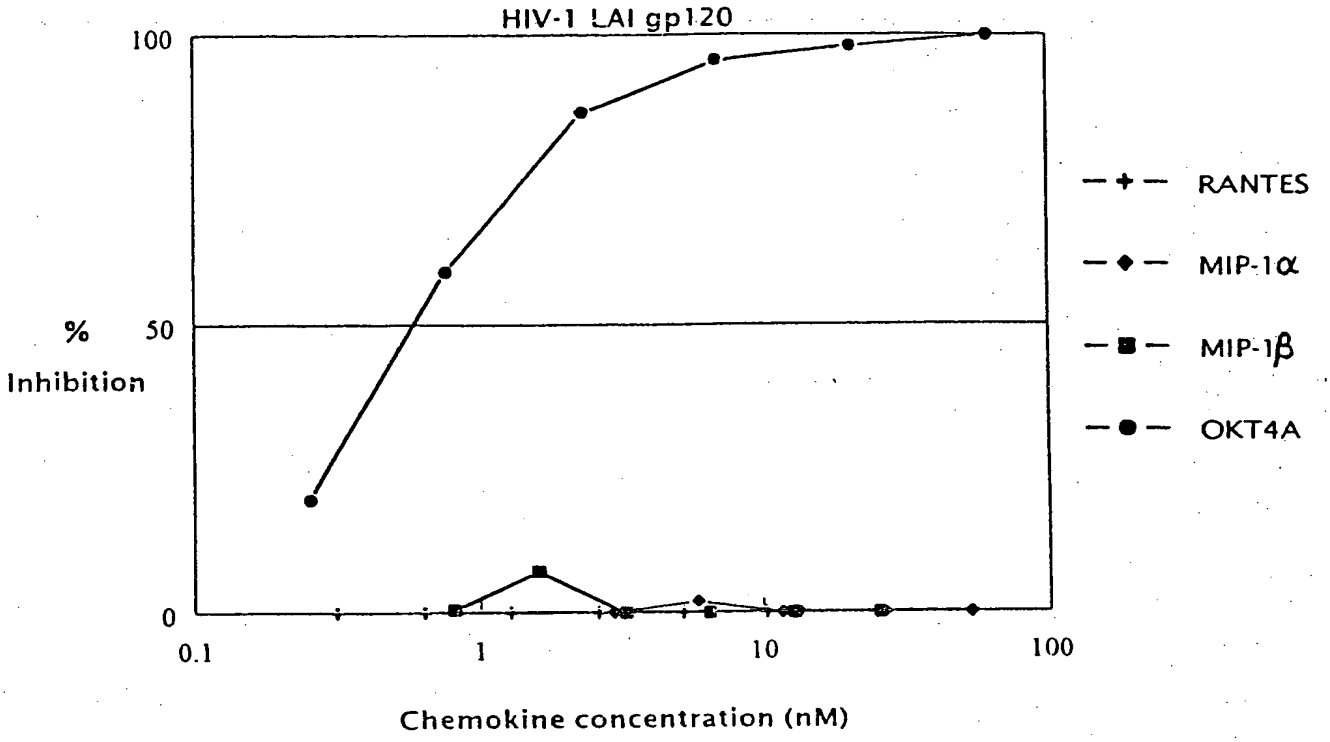
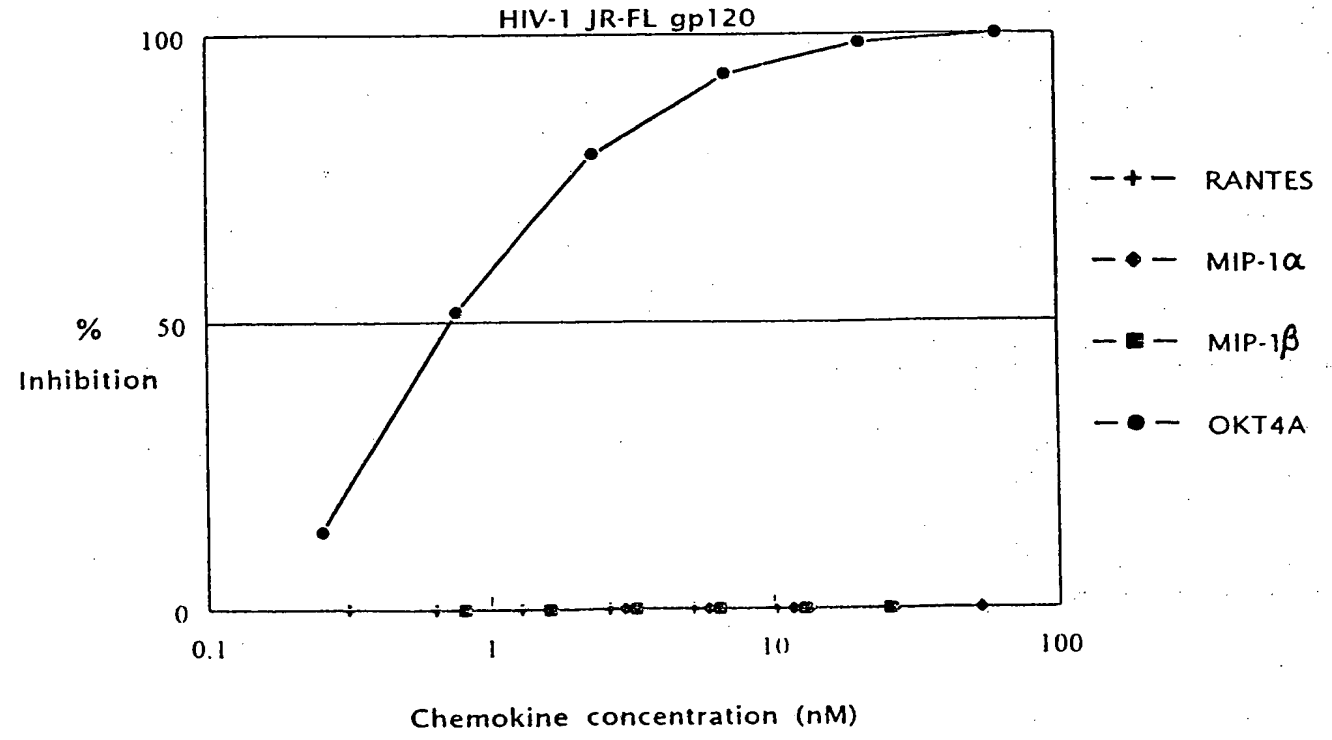

HIV-1 LAI gp120
100
50
0
% Inhibition
0.1
1
10
100
Chemokine concentration (nM)
RANTES
MIP-1α
MIP-1β
OKT4A

FIG. 2B


HIV-1 JR-FL gp120
100
50
0
% Inhibition
0.1
1
10
100
Chemokine concentration (nM)
RANTES
MIP-1α
MIP-1β
OKT4A